APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1978, p. 439-443
0099-2240/78/0035-0439$02.00/0

Vol. 35, No. 2
Printed in U.S.A.

Colonial Variation, Capsule Formation, and Bacteriophage Resistance in *Bacteroides thetaiotaomicron*

SHARON BURT,[1] SHEILA MELDRUM,[1] D. R. WOODS,[1]* AND D. T. JONES[2]

Department of Microbiology, Rhodes University,[1] Grahamstown, 6140, and Biology Department, University of Natal,[2] Durban, South Africa

Received for publication 7 June 1977

A *Bacteroides thetaiotaomicron* strain segregated two unstable colonial variants at high frequency. There is a correlation between colony morphology, encapsulation, Giemsa staining, and bacteriophage resistance.

Prévot and Fredette (13) classified all the anaerobic gram-negative rod bacteria that formed capsules as the genus *Capsularis*. Holdeman and Moore (5, 6, 12) after an extensive reexamination of the classification of anaerobic bacteria classified gram-negative, nonmotile, rod-shaped bacteria without spores as *Bacteroides fragilis*. This group is further characterized by the production of large amounts of succinic and acetic acids, but no butyric acid. Their growth is stimulated by the addition of 20% bile. The *B. fragilis* subsp. *thetaiotaomicron* was included in *B. fragilis*, but the species rank has recently been reinstated as a result of deoxyribonucleic acid homology and serological studies (2). Certain *Bacteroides* strains have been shown to be encapsulated, and Kasper (8) suggested that capsular antigens may form the basis of a serogrouping system in *B. fragilis*.

We isolated a *Bacteroides* strain from a human fecal sample that was identified as *B. thetaiotaomicron* and confirmed by L. V. Holdeman, Virginia Polytechnic Institute and State University Anaerobic Laboratory, Blacksburg, Va. The strain proved to be interesting because it was unstable and segregated two colonial variants at high frequencies. The one colony type was characterized by being circular, entire, greyish, semitranslucent, and like a fried egg in appearance. The second was circular, entire, white, opaque, mucoid, and convex in appearance. The white colony type was slightly larger than the grey variant.

The anaerobic glove box and technique described by Moodie and Woods (11) were used for the maintenance and cultivation of both colony types on brain heart infusion agar (1). Both colony types were unstable and always gave some colonies of the opposite colony type. In our experiments, 100 colonies of each type were

TABLE 1. *Frequency of segregation of variant cell types at 37 and 42°C*[a]

Colony type	Segregation frequency (variant/cell/generation)	
	37°C	42°C
Grey variant colonies from white clone	1.1×10^{-2}	1×10^{-3}
White variant colonies from grey clone	1.4×10^{-2}	3×10^{-3}

[a] Variant cell types from each colony type were determined by serial plating of broth cultures grown at 37°C and 42°C for 18 h.

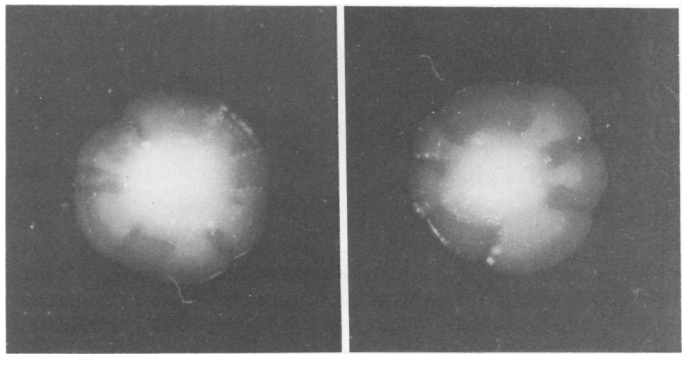

FIG. 1. *Grey colonies showing white pie sectors.*

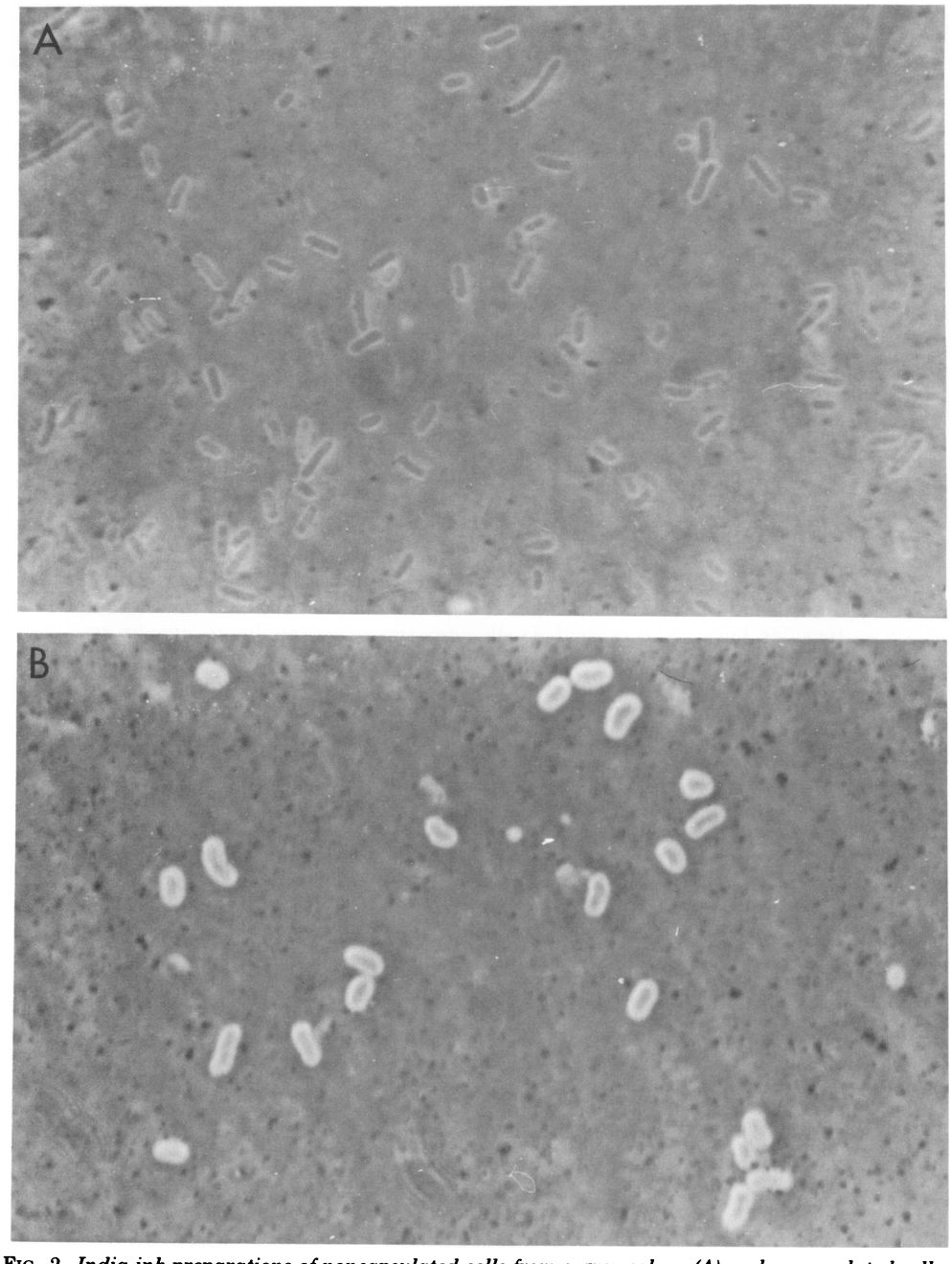

FIG. 2. *India ink preparations of noncapsulated cells from a grey colony (A) and encapsulated cells from a white colony (B).*

picked and streaked on brain heart infusion agar. After incubation, each streak plate contained some colonies of the opposite colony type. Stable colony types were not obtained even after many serial clonings or after treatment with mutagens (*N*-methyl-*N*-nitro-nitrosoguanidine and ultraviolet light). The proportion of white colony type cells within a grey colony type and vice versa was determined by suspending each colony in 10 ml of anaerobic buffer and immediately serial plating on brain heart infusion agar. The results indicated that each colony type con-

tained about 12% of cells that gave rise to the other colony type. The frequency of segregation of variant cell types arising from each colony type was determined by serial plating of broth cultures grown at 37 and 42°C for 18 h. The frequency of segregation at 37°C was between 1.1×10^{-2} and 1.4×10^{-2} variants/cell per generation (Table 1). This segregation frequency was reduced approximately 10-fold by growth at 42°C. The instability and high frequency of seg-

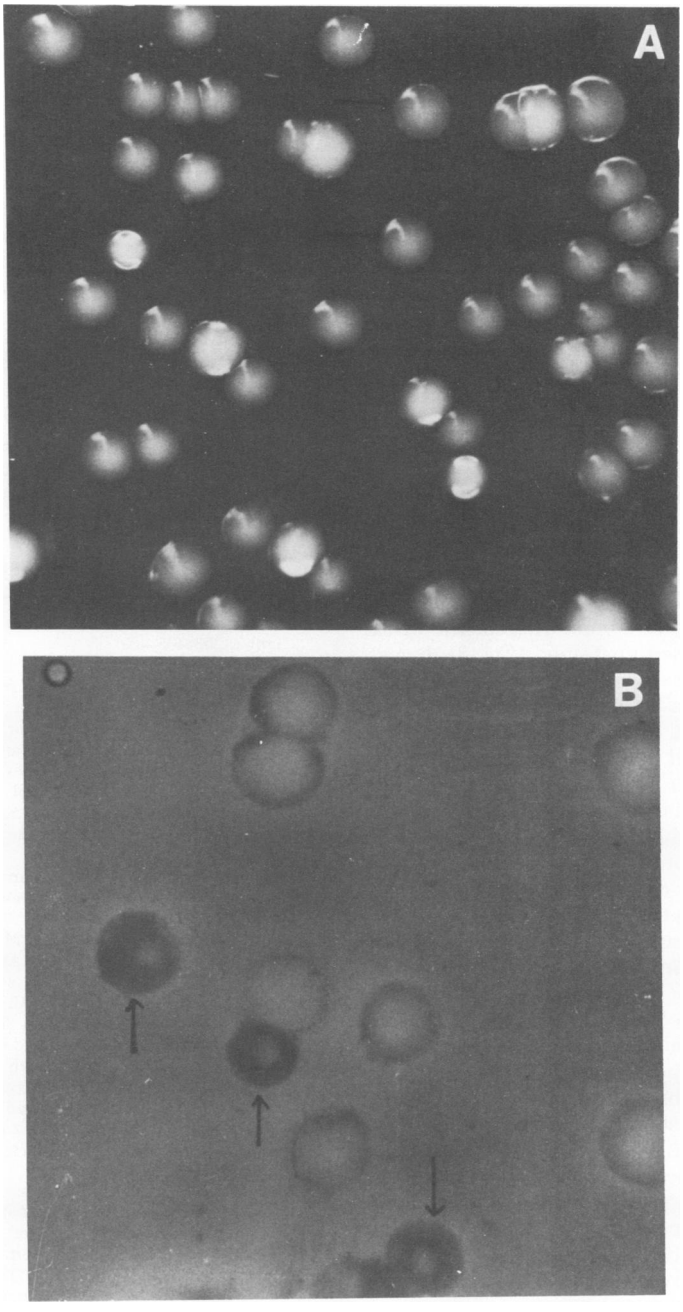

FIG. 3. *Unstained (A) and Giemsa-stained (B) colonies: arrows indicate the grey colony types that are unstained in (A) but stained in (B).*

regation resulted in the formation of pie sectors within many colonies (Fig. 1). The formation of pie sectors and the high segregation frequencies could indicate control by cytoplasmic genes. Growth of cells in media containing acriflavine, ethidium bromide, or sodium dodecyl sulfate, which enhance the segregation of plasmid genes in *Escherichia coli*, did not affect the colonial segregation frequencies. Although these results suggest that plasmids are not involved, many plasmids, especially those with a high molecular weight, are resistant to removal by curing agents. However, the fact that segregants always include the two colony types at the same frequencies indicates that the loss of a plasmid is not responsible for the high segregation frequencies.

Morphological studies on the cells from the two colony types indicated that cells from the grey colony type were not encapsulated, whereas cells from the white colony were. The capsules were observed by phase-contrast microscopy of India ink preparations (Fig. 2). Growth of the white colony type on peptone-yeast extract-sucrose medium (5) enhanced the production of the capsule.

The colony types could be further distinguished by staining with Giemsa stain (Fig. 3). They did not stain immediately after removal from anaerobic conditions and required 3 days of exposure to air before staining. The grey colony type stained dark blue, whereas the white colony type remained unstained (Fig. 3). It was also possible to observe pie sectors of Giemsa-stained cells within a predominantly white colony and vice versa. The Giemsa staining technique has been used to isolate nuclease-deficient mutants of *E. coli* (10), but no difference in desoxyribonuclease or ribonuclease activity was detected between the two *B. fragilis* subsp. *thetaiotaomicron* colony types before or after exposure to air for 3 days. Nuclease activity was assayed by the method of Gesteland (3). The presence of a capsule in cells from the white colony could account for the cells not staining with Giemsa stain. However, the reason why cells from the grey colony only stained dark blue after exposure to air for 3 days is not known. These cells do not possess an unstable nuclease.

A phage, B1, which was specific for the *B. thetaiotaomicron* strain, was isolated from a sewage anaerobic digester (S. J. Burt and D. R. Woods, J. Gen. Microbiol., in press). Overnight cultures of 100 colonies of each colony type were tested for resistance to phage B1 by spotting high titer lysates (10^{10} plaque-forming units per ml) onto soft agar overlays seeded with the cells of each colony. The grey, noncapsulated variant was sensitive to the phage B1, but the white, encapsulated strain was resistant to the phage. The correlation between phage resistance, encapsulation, and colony type is similar to that reported for *Clostridium perfringens* by Grant and Reiman (4), who described mutants with surface alterations that occurred spontaneously in stock cultures of *C. perfringens*. Jones et al. (7) also reported a correlation between colony morphology and phage sensitivity in *Brucella abortus*. Variations in colony morphology have been observed for *Neisseria gonorrhoea* (9), *Vibrio cholera* (14), and *B. nodusus* (15).

Observations with 35 other *Bacteroides* strains indicate that this unstable segregation of encapsulation is a widespread characteristic within the genus. These results support the observations of Kasper (8) on the presence of a capsule in *Bacteroides* strains, but the segregation phenomenon should be borne in mind when using capsular antigens for serogrouping. In our experience, the production of capsules is enhanced on peptone-yeast extract-sucrose medium (5), and studies have been initiated to investigate the control of capsule formation.

D.R.W. acknowledges a research grant from the South African Medical Research Council.

LITERATURE CITED

1. **Burt, S. J., and D. R. Woods.** 1976. R factor transfer to obligate anaerobes from *Escherichia coli*. J. Gen. Microbiol. **93**:405–409.
2. **Cato, E. P., and J. L. Johnson.** 1976. Reinstatement of species rank for *Bacteroides fragilis, B. ovatus, B. distasonis, B. thetaiotaomicron*, and *B. vulgatus*: designation of neotype strains for *Bacteroides fragilis* (Veillon and Zuber) Castellani and Chalmers and *Bacteroides thetaiotaomicron* (Distaso) Castellani and Chalmers. Int. J. Syst. Bacteriol. **26**:230–237.
3. **Gesteland, R. F.** 1966. Isolation and characterisation of ribonuclease I mutants of *Escherichia coli*. J. Mol. Biol. **16**:67–84.
4. **Grant, R. B., and H. P. Reiman.** 1976. Temperate phages of *Clostridium perfringens* type C. Can. J. Microbiol. **22**:603–610.
5. **Holdeman, L. V., and W. E. C. Moore.** 1972. Anaerobe laboratory manual. V.P.I. Anaerobe Laboratory, Blacksburg, Va.
6. **Holdeman, L. V., and W. E. C. Moore.** 1974. Gram-negative anaerobic bacteria, p. 384–426. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
7. **Jones, L. M., C.R. McDuff, and J. B. Wilson.** 1962. Phenotypic alterations in the colonial morphology of *Brucella abortus* due to the bacteriophage carrier state. J. Bacteriol. **83**:860–866.
8. **Kasper, D.** 1976. The polysaccharide capsule of *Bacteroides fragilis* subspecies *fragilis*: immunochemical and morphologic definition. J. Infect. Dis. **133**:79–87.
9. **Kellogg, D. S., Jr., I. R. Cohen, L. C. Norins, A. L. Schroeter, and G. Reising.** 1968. *Neisseria gonorrhoeae*. II. Colonial variation and pathogenicity during 35 months in vitro. J. Bacteriol. **96**:596–605.
10. **Miller, J. H.** 1972. Experiments in molecular genetics.

Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

11. **Moodie, H. L., and D. R. Woods.** 1973. Isolation of obligate anaerobic faecal bacteria using an anerobic glove cabinet. S. Afr. Med. J. **47**:1739–1742.
12. **Moore, W. E. C., and L. V. Holdeman.** 1972. Identification of anaerobic bacteria. Am. J. Clin. Nutr. **25**:1306–1313.
13. **Prévot, A., and V. Fredette.** 1966. Manual for the classification and determination of the anaerobic bacteria. Lea & Febiger, Philadelphia.
14. **Salles, C. A., S. Voros, E. C. Marbell, and L. Amenuvor.** 1976. Colony morphology of *Vibrio cholera* on SV medium. J. Appl. Bacteriol. **40**:213–216.
15. **Thorley, C. M.** 1976. A simplified method for isolation of *Bacteroides nodusus* from bovine foot-rot and studies on its colony morphology and serology. J. Appl. Bacteriol. **40**:301–309.